corresponding to position 348 of SEQ ID NO: 2; (b) a histidine residue corresponding to at least one of positions 238, 252, 347, 440, 451, and 579 of SEQ ID NO: 2; and (c) a heparin-binding sequence residue corresponding to at least one of positions 446-451 of SEQ ID NO: 2.

REMARKS

Claims 30-34 and 46-49 are pending. Claims 30 and 46 have been amended. The claims as amended now recite methods of using a modified heparinase II molecule having at least one amino acid residue substitution from a native heparinase II molecule. The modified heparinase II molecules having specific residue substitutions fall under the broad category of "modified heparinase II" molecules that has been under examination in the pending claims. The specific residues were also recited in the claims as filed prior to the restriction requirement. In particular, support for the amendments is found in original claims 13 and 52 as well as in the specification on pages 5, 14-16, 19-20 and in Examples 20 and 21.

Applicants are also filing herewith a Request for Continued Examination to provide the Examiner with a sufficient amount of time to consider this Amendment. No new matter has been added.

Interview with Examiner Hutson

Applicants wish to thank Examiner Hutson for his courtesy in granting and conducting a telephone interview with Applicants' representatives on November 7, 2002. In the interview, issues relating to the rejections under 35 U.S.C. §112, first paragraph, 35 U.S.C. §103 and §102(a) were discussed. The details of these discussions are provided below under each section.

Claim Objections

Independent claims 30 and 46 and dependent claim 49 were objected to by the Examiner as their format appeared to be confusing and unclear. The Examiner suggested that a colon be added to the end of the second paragraph in claims 30 and 46. Applicants have amended independent claims 30 and 46 as suggested by the Examiner. The correction of the typographical error has no effect on the scope of the claims. Claim 49, which was rejected as depending from claim 46 remains unamended.

Applicants respectfully request that the Examiner withdraw the objection of claims 30, 46 and 49 in view of the claim amendments.

Rejections Under 35 U.S.C. §112, first paragraph

Claims 30, 33, 34, 46, 47 and 48 have been rejected under 35 U.S.C. §112, first paragraph, as containing subject matter not described in the specification in a way to convey to one skilled in the art that the Applicants, at the time the application was filed, had possession of the claimed invention. The Examiner maintains that the identification of important amino acid residues in the catalytic and binding domains of heparinase II as well as the limited number of species is insufficient "...to describe the claimed invention in such full, clear, concise, and exact terms..." that one of skill would recognize the Applicants' possession of the claimed invention. Applicants' prior arguments were rejected because according to the Examiner the claimed enzymes were limited only by their function and not their structure.

Claims 30, 33, 34, 46, 47 and 48 have also been rejected under 35 U.S.C. §112, first paragraph as the specification does not enable one of skill in the art to make and use the invention commensurate in scope with these claims. The Examiner maintains that while variants of a known sequence are produced routinely in the art, guidance is not provided for the selection of which of the variants have the claimed property. It is concluded that in order to practice the claimed invention, undue experimentation would be required as an infinite number of variants would need to be produced and tested.

Claim Amendments and Interview with the Examiner

Claims 30 and 46 have been amended to recite a modified heparinase II having specific amino acid residue substitutions that result in the modified heparinase II having a modified product profile and/or modified k_{cat} for use in the claimed methods. The modified heparinase II molecules may also have additional substitutions of residues outside the enzyme's active and/or binding sites. Thus, the claims now recite structural limitations which have been identified according to the invention to produce the functional properties of the claimed enzymes. The claim amendments were discussed with Examiner Hutson in the interview. The Examiner agreed that the addition of structural limitations may be helpful in overcoming the rejection of these claims.

Rejection under 112 for Lack of Possession of the Invention

The disclosure of the amino acid residues that play a role in the enzymatic and binding activity of heparinase II along with the number of described species is sufficient to demonstrate Applicants were in possession of the claimed invention.

In the specification, several representative species are disclosed which include modified heparinase II molecules with mutations at Cys 348, His 252, His 347, His 440, His 238, His 451 and His 579. These modified heparinase II enzymes exhibit differential enzymatic activity toward heparin or heparan sulfate or both. For example, mutation of Cys 348 resulted in a modified heparinase II enzyme with decreased enzymatic activity toward heparin by reacting exclusively on heparan sulfate. The His 440 mutant is less active towards heparin but has the same activity toward heparan sulfate. His 451, 238 and 579 mutants have reduced activity towards both heparin and heparan sulfate.

The disclosure also provides the amino acid residues important in the enzymatic and binding activity of heparinase II. The data presented provides that the binding pocket of heparinase II includes two active sites, one which contains Cys 348 and cleaves heparin and one that does not contain Cys 348 and cleaves heparan sulfate. Another active site residue, His 451, which plays a key role in the degradation of heparin, is also provided. His 451 as well as His 238 and 579 are shown to be important for heparinase II activity. His 252, 347 and 440 are shown to be important in substrate binding. Additionally, residues 446-451 are provided as a heparin-binding sequence important in the control of the activity of heparinase II with respect to heparin.

Based on the foregoing, the specification provides an adequate description of the invention to demonstrate that Applicants had possession of the claimed invention as well as adequate information about the structure of heparinase II as it relates to the function of the enzyme.

Therefore, Applicants respectfully request that the rejection of claims 30, 33, 34, 46, 47 and 48 under 35 U.S.C. §112, first paragraph be withdrawn in view of the arguments presented above.

Rejections under 112 for lack of Enablement

As amended, the claims provide a set of residues that can be modified to produce molecules with altered activity. The recitation of these residues in combination with the

teachings of the disclosure and extensive working examples provides adequate guidance to one of skill in the art to determine which modification would reasonably be expected to produce a modified molecule with the desired activity. The specification, working examples and level of skill in the art provide sufficient guidance for one of ordinary skill to identify, make and test the modified heparinase II molecules and, therefore, use the molecules as stated in the amended claims, with only routine experimentation.

One of skill in the art is able to identify heparinase II molecules useful according to the claimed methods and subsequently make modified heparinase II molecules with the teachings provided by Applicants' specification and the high level of knowledge in the art. For example, the specification provides the nucleic acid sequence of a heparinase II molecule (SEQ ID NO: 1) and the predicted amino acid sequence of the polypeptide it encodes (SEQ ID NO: 2).

One of skill is sufficiently enabled to produce modified heparinase II molecules with standard techniques known in the art and the guidance provided in Applicants' specification through the identification of the residues that are important to the function of the enzyme. An adequate and extensive description of the catalytic and binding sites of heparinase II is provided. Through the description of the essential residues of the catalytic and binding sites, the specification provides the structure of heparinase II in relation to its function towards heparin and/or heparan sulfate. The amended claims also recite these important residues. In addition, the specification provides a representative number of species that demonstrate the various mutations which alter the enzymatic activity of the enzyme. One of skill would be able to produce modified heparinase II molecules with altered activity due to the recitation of the specific residues to be substituted in the amended claims as well as the description, working examples and lists of substitutions provided in the specification.

Techniques for determining modified product profiles and/or k_{cat} values are well known in the art, and examples of these techniques are also given in the specification (e.g. pages 18, 21 and 22). For example, a method of using mass spectrometry and capillary electrophoresis is described for determining the product profile of a heparinase. Other methods provided for determining product profiles rely on viscosity, total UV absorbance or mass spectrometry or capillary electrophoresis alone. Enzymatic activity assays for determining the k_{cat} value of a heparinase enzyme are also described in the specification. In view of this, one of skill would be required to perform only routine experimentation to screen modified heparinase II enzymes having the structures recited in the claims.

A reasonable amount of guidance is provided for the selection of the modified heparinase II enzymes which exhibit the claimed properties. Because of Applicants' description of mutant heparinase II enzymes as well as the essential residues of the catalytic and binding sites, the disclosure provides a clear finite set of possible modifications of heparinase II. In addition, the claims as amended clearly recite the residues that can be substituted in order to alter the enzyme's activity. Applicants further maintain that the specification also provides sufficient guidance for testing the modified heparinase II enzymes produced to select those with the claimed properties. The specification provides sufficient guidance to one of ordinary skill in the art make and use the invention commensurate in scope with these claims.

Applicants, therefore, respectfully request the Examiner withdraw the rejections under 35 U.S.C. §112, first paragraph.

Rejections under 35 U.S.C. §102(a)

As discussed in the Interview with the Examiner, inventors Liu and Venkataraman contributed to the claims encompassing heparinase I and not the claims encompassing heparinase II. Sasisekharan and Shriver alone are the correct inventors of the heparinase II claims. Independent claim 30 is directed to methods of using modified heparinase I and II. Due to a species election in the restriction requirement, modified heparinase I has been restricted from claim 30. However, in the event a generic claim is allowed, Applicants will be entitled to consideration of the modified heparinase I species. The declaration of Dr. Ram Sasisekharan under 37 C.F.R. §1.1.32 submitted previously (March 25, 2002) states the inventorship in regard to both heparinse I and heparinase II. In addition, the declaration correctly described co-author Yini Hu as merely working under the direction of Dr. Sasisekharan but not contributing to the conception of the invention. The paper by Shriver et al. (1998) is not "by another" and does not anticipate claims 30, 31, 33, 46 and 47 under 35 U.S.C. §102(a).

Based on this clarification, the Examiner is respectfully requested to withdraw the rejection under 35 U.S.C. §102(a).

Rejections under 35 U.S.C. §102(e)

Claims 30, 33, 46 and 47 have been rejected under 35 U.S.C. §102(e) as being anticipated by Su et al (U.S. Patent No. 5,681,733). The Examiner maintains that the use of heparinase I and

III, which have a modified product profile relative to native heparinase II, anticipates claims 30, 33, 46 and 47.

The claims as amended are not anticipated by the teachings of Su et al. The modified heparinase II enzymes in the claimed methods require that at least one of a list of specific amino acid residues is modified compared to a native heparinase II. These modified heparinase II molecules do not include native heparinase I, II or III and are not disclosed by Su et al.

The Applicants respectfully request the Examiner withdraw the rejection to claims 30, 33, 46 and 47 under 35 U.S.C. §102(e).

Rejections under 35 U.S.C. §103(a)

Claims 34 and 48 are rejected under 35 U.S.C. §103(a) as being unpatentable over Su et al. and further in view of Langer et al. (U.S. Patent No. 4,373,023). The Examiner maintains that heparinases I and III taught by Su et al. meet the functional limitations of the heparinases of the claimed methods and that, in combination with Langer et al., would have rendered the claimed methods obvious.

The claims were not obvious in view of Su et al. and Langer et al. because Su et al. does not describe the claimed modified heparinase II molecules. As discussed above, the claims recite specific amino acid residues that can be modified to produce the heparinase II molecules with altered activity for use in the claimed methods. Thus, the combination of references does not produce all of the claimed elements.

Applicants respectfully request that the Examiner withdraw the rejection of the claims in light of the claim amendments and the foregoing arguments.

CONCLUSION

In view of the foregoing amendments and remarks, this application should now be in condition for allowance. A notice to this effect is respectfully requested. If the Examiner believes, after this amendment, that the application is not in condition for allowance, the Examiner is requested to call the Applicants' attorney at the telephone number listed below.

Respectfully submitted,
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MARKED-UP CLAIMS

30. (Twice Amended) A method of specifically cleaving a heparin-like glycosaminoglycan, comprising:

contacting a heparin-like glycosaminoglycan with the heparinase of any one of:

a substantially pure heparinase comprising a modified heparinase II having a modified product profile, wherein the modified product profile of the modified heparinase II is at least 10% different than a native product profile of a native heparinase II,

a substantially pure heparinase comprising a modified heparinase II that can cleave a glycosaminoglycan substrate having a modified heparinase II k_{cat} value, wherein the modified heparinase II k_{cat} value is at least 10% different than a native heparinase II k_{cat} value, and

a substantially pure heparinase comprising a modified heparinase I wherein the modified heparinase I has enzymatic activity that is not dependent on the presence of calcium,

wherein the modified heparinase II contains at least one amino acid residue that has been substituted with a different amino acid than in native heparinase II and wherein the residue that has been substituted is selected from the group consisting of (a) a cysteine residue corresponding to position 348 of SEQ ID NO: 2; (b) a histidine residue corresponding to at least one of positions 238, 252, 347, 440, 451, and 579 of SEQ ID NO: 2; and (c) a heparin-binding sequence residue corresponding to at least one of positions 446-451 of SEQ ID NO: 2,

and wherein the modified heparinase I contains at least one amino acid residue that has been substituted with a different amino acid than in native heparinase I and wherein the residue that has been substituted is a serine residue corresponding to position 377 of SEQ ID NO: 4.

46. (Twice Amended) A method of specifically cleaving a heparan sulfate-like glycosaminoglycan, comprising:

contacting a heparan sulfate containing fluid with the heparinase of any one of:

a substantially pure heparinase comprising a modified heparinase II having a modified product profile, wherein the modified product profile of the modified heparinase II is at least 10% different than a native product profile of a native heparinase II and

a substantially pure heparinase comprising a modified heparinase II that can cleave a glycosaminoglycan substrate having a modified heparinase II k_{cat} value, wherein the modified heparinase II k_{cat} value is at least 10% different than a native heparinase II k_{cat} value,

wherein the modified heparinase II contains at least one amino acid residue that has been substituted with a different amino acid than in native heparinase II and wherein the residue that has been substituted is selected from the group consisting of (a) a cysteine residue corresponding to position 348 of SEQ ID NO: 2; (b) a histidine residue corresponding to at least one of positions 238, 252, 347, 440, 451, and 579 of SEQ ID NO: 2; and (c) a heparin-binding sequence residue corresponding to at least one of positions 446-451 of SEQ ID NO: 2.